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Tumor necrosis factor-α during continuous high-flux hemodialysis in sepsis with acute renal failure

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Tumor necrosis factor-α during continuous high-flux hemodialysis in sepsis with acute renal failure. Suppressed *ex vivo* endotoxin (ET)-induced production of the proinflammatory cytokine, tumor necrosis factor-α (TNF-α), in isolated mononuclear cells (PBMCs) is associated with fatal outcome in severe sepsis. PBMCs from surviving patients, but not those from nonsurviving patients, recover their capacity to produce normal amounts of TNF-α. We tested the influence of two modalities of continuous renal replacement therapy (CRRT) on *ex vivo*-induced whole-blood production of TNF-α and inhibitory TNF-soluble receptor type I (TNFsRI) in 12 patients with acute renal failure and sepsis (APACHE II score 22 to 30).

Methods. Standard continuous venovenous hemofiltration (CVVH; 36 liters of bicarbonate substitution fluid per day) was performed in 7 patients using polyamid hemofilters (FH66; Gambro). In an additional five patients, we performed daily 18 hours of high-flux hemodialysis (CHFD) using polysulfon F60S dialyzers (Fresenius) and 75 liters of bicarbonate dialysate using the GENIUS® single-pass batch dialysis system. Samples were separated from the blood circuit as well as from the ultrafiltrate/spent dialysate lines at the start, during, and end of treatment. Whole-blood samples were incubated with 1 ng/ml of ET for three hours at 37°C. Ultrafiltrate or dialysate samples were incubated with donor whole blood in the presence of ET to measure suppressing activity in ultrafiltrate and spent dialysate.

Results. At the start of CRRT, ET-induced whole-blood TNF-α production was suppressed to approximately 10% of that in normal controls. During CVVH, median ET-induced TNF-α production increased from 0.35 ng/ml at the start to 1.2 ng/ml at three hours, but decreased to pre-CVVH levels at the end of a 24-hour period. In contrast, in patients on CHFD, the median ET-induced TNF-α production was 0.5 ng/ml at the start, 1.1 ng/ml at 3 hours, 1.6 ng/ml at six hours, and 1.5 ng/ml at the end of 18 hours of treatment. The ultrafiltrate obtained after three hours of CVVH did not contain suppressing activity. In CHFD, the spent dialysate as compared with fresh dialysate suppressed ET-induced TNF-α production in donor blood by 33% throughout the 18 hours of treatment. Whole-blood production of TNFsRI did not change significantly at any time point during CVVH or CHFD.

Conclusion. These data suggest that high-volume CHFD is superior to standard CVVH in removing a suppressing factor of proinflammatory cytokine production. As CVVH only transiently improves TNF-α production, it is most likely that the putative suppressing factor is removed because of saturable membrane adsorption in CVVH. In CHFD, there is a combination of adsorption and detectable diffusion into the dialysate. It remains to be shown whether a further increase in the volume of dialysate per day is able to not only improve but normalize the cytokine response and improve outcome in septic patients with acute renal failure.

Key words: renal replacement therapy, cytokines, nephrotoxicity, sepsis, acute renal failure, MODS.

Multiple organ dysfunction syndrome (MODS) is the most common cause of acute renal failure in the intensive care unit today. There is no doubt that proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β play an important role in the induction and continuation of severe disturbances in patients suffering from sepsis. However, there is no correlation between plasma levels of TNF-α, IL-1β, and IL-6 and the degree of activation of circulating mononuclear cells (PBMCs), as indicated by the cell-associated concentrations of the same cytokines in PBMCs during sepsis [1]. In contrast to cytokine plasma levels, which are generally elevated in septic patients, it has been shown that cell-associated cytokines in PBMCs may be decreased and that the capacity of PBMCs to produce TNF-α and IL-1β in response to endotoxin (ET) *in vitro* is significantly reduced, particularly in patients with gram-negative sepsis [2, 3]. Astiz et al showed that the reduced capacity of PBMC production of proinflammatory cytokines was associated with increased plasma levels of IL-10 and prostaglandin E_2, which are known to inhibit the production of proinflammatory cytokines [3]. The authors concluded that in severe sepsis, there may be a hyporesponsiveness of PBMCs because of an overproduction of anti-inflammatory cytokines. Munoz et al demonstrated that PBMCs from surviving patients, but not those from nonsurviving patients, recover their capacity to produce normal amounts of TNF-α and IL-1β [2]. It may be concluded from these data that interventions that are able to improve the capacity of PBMCs to produce proinflammatory cytokines

could be beneficial with respect to the outcome in patients suffering from severe sepsis. We investigated the effect of two modalities of continuous renal replacement therapy (CRRT) on *ex vivo* ET-induced cytokine production in patients with acute renal failure caused by MODS.

METHODS

Twelve patients (8 men and 4 women) in the intensive care unit, all on mechanical ventilation and with acute renal failure in the course of MODS, were studied. The underlying diseases included pancreatitis, esophagus carcinoma, pneumonia, bowel obstruction with perforation, endocarditis, and liver failure. The APACHE II score was 22 to 30 in all patients.

Group A

In seven patients (5 men and 2 women), continuous venovenous hemofiltration (CVVH) was performed using polyamide ultrafilters (FH66; Gambro), bicarbonate-buffered substitution fluid, a blood flow of 100 ml/min, and an ultrafiltration rate of 25 ml/min (equal to 1500 ml/hr and 36 liter/day). Samples were taken from the afferent blood line of the extracorporeal circuit as well as from the ultrafiltrate line 5 minutes, 3 hours, and 24 hours after a new filter was connected.

Group B

In five patients (3 men and 2 women), continuous high-flux hemodialysis (CHFD) was performed with polysulfone dialyzers (F60S; Fresenius) and acetate-free bicarbonate-buffered dialysate using the GENIUS® single-pass batch hemodialysis system. In this system, 75 liters of sterile dialysate were recirculated single pass in a closed loop dialysate circuit. Blood flow was 70 ml/min and equal to a countercurrent dialysate flow of 70 ml/min (4200 ml/hr, 75 liter/18 hr). Samples were taken from the afferent blood line as well as from the fresh and the spent dialysate lines at 5 minutes, 3 hours, 6 hours, and 18 hours of CHFD.

Whole-blood samples (1 ml) were incubated with equal amounts of cell culture medium (RPMI) containing 1 ng/ml of ET (final concentration) for three hours at 37°C in pyrogen-free polypropylene tubes. Following incubation, tubes were centrifuged, and whole-blood culture supernatants were separated to measure cytokines [TNF-α, TNF-soluble receptor type I (TNFsRI), IL-10] using specific immunoassays.

In addition, ultrafiltrate samples in CVVH and dialysate samples (fresh and spent) in CHFD were incubated with donor blood in the presence of 1 ng/ml ET for three hours at 37°C in order to test for suppressing activity of ET-induced whole blood cytokine production. TNF-α was measured by specific immunoassays in whole blood culture supernatants.

RESULTS

Ex vivo ET-induced whole-blood production of TNF-α was significantly suppressed in patients with acute renal failure and sepsis ($N = 10$) compared with blood samples from healthy controls ($N = 18$; Fig. 1). The median values were 500 pg/ml in patients and 7100 pg/ml in controls. In contrast, the production of the anti-inflammatory cytokine IL-10 was slightly increased in whole-blood samples of patients compared with controls (Fig. 1).

During CVVH, suppressed ET-induced TNF-α production improved transiently from a median value of 0.34 ng/ml (range 0.23 to 0.72) at 5 minutes to 1.2 ng/ml (0.55 to 1.40) after 3 hours and returned to 0.51 ng/ml (0.34 to 1.09) after 24 hours (Fig. 2). During CHFD, TNF-α production improved continuously from 0.49 ng/ml (0.10 to 1.80) at 5 minutes to 1.1 ng/ml (0.23 to 2.3) at 3 hours, to 1.59 (0.49 to 2.8) at 6 hours, and to 1.52 ng/ml (0.76 to 2.42) at 18 hours (Fig. 2). In contrast, ET-induced whole-blood production of the inhibitory soluble TNF receptor type I (TNFsRI) did not change. During CVVH, TNFsRI production was 9.0 ng/ml (4.3 to 11.3) at 5 minutes, 8.0 ng/ml (4.03 to 12.0) at 3 hours, and after 24 hours, it was 8.5 ng/ml (6.4 to 11.6). During CHFD, ET-induced whole blood production of TNFsRI was 8.0 ng/ml (4.4 to 12) at five minutes, 8.8 ng/ml (4.1 to 11.6) at 3 hours, 9.0 ng/ml (3.7 to 12.8) at 6 hours, and 7.8 ng/ml (3.7 to 11.0) after 18 hours (Fig. 3).

Compared with isotonic saline as the control, the ultrafiltrate samples obtained after five minutes and three hours of CVVH did not significantly suppress ET-induced TNF-α production in donor blood. In contrast, spent dialysate compared with fresh dialysate suppressed TNF-α production in donor blood by 33 ± 7% throughout the 18 hours of CHFD.

DISCUSSION

Our data confirm previous publications describing severe suppression of the production of proinflammatory cytokines in circulating mononuclear cells during sepsis [2, 3]. In addition, we show that the synthesis of proinflammatory cytokines, but not that of anti-inflammatory cytokines, is suppressed. The data for TNF-α and TNFsRI production are shown, and similar results were obtained for proinflammatory IL-1β, which was suppressed, and inhibitory IL-1 receptor antagonist, which was not suppressed. Also, plasma levels of IL-10 as well as ET-induced whole-blood production of IL-10 did not change during CVVH or CHFD (data not shown). We conclude that CRRT exclusively modifies the capacity of circulating cells to produce proinflammatory cytokines without influencing the synthesis of anti-inflammatory mediators in patients with severe sepsis. Standard CVVH with a

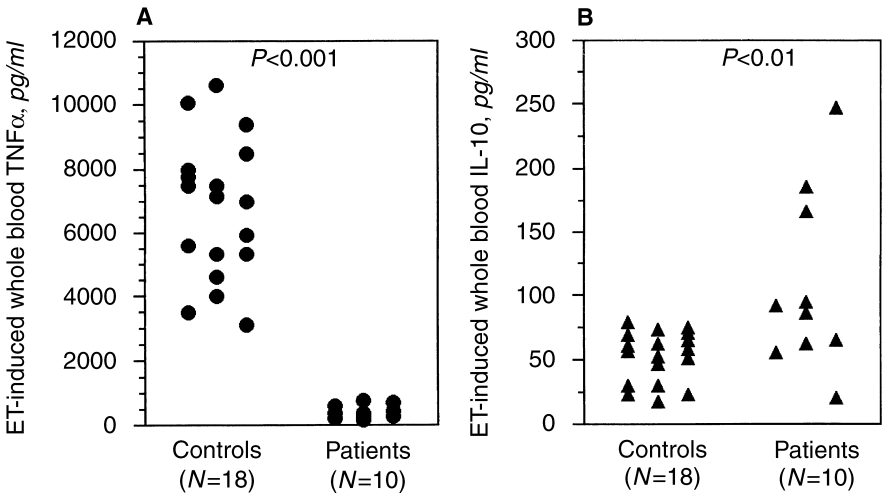

Fig. 1. Endotoxin-induced whole blood production of tumor necrosis factor-alpha (*A*; TNF-α) and interleukin-10 (*B*; IL-10) in healthy controls (*N* = 18) and patients with sepsis and acute renal failure.

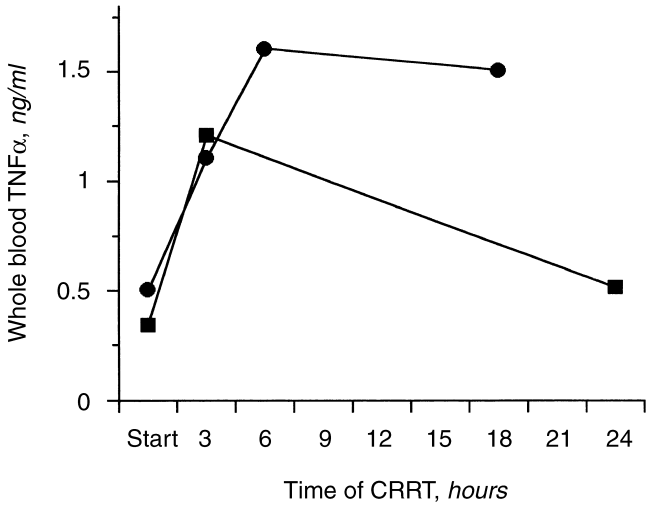

Fig. 2. Effect of continuous venovenous hemofiltration (■; CVVH, 1.5 liters/hr) and continuous high flux hemodialysis (●; GENIUS®-CHFD, 4.2 liters/hr) on *ex vivo* whole-blood TNF-α production in septic patients with acute renal failure. Symbols indicate the medians of *N* = 7 for CVVH and *N* = 5 for CHFD.

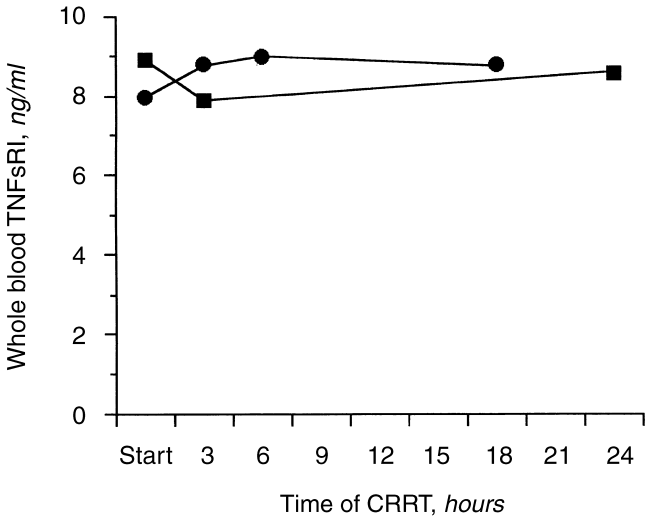

Fig. 3. Effect of CVVH (■; 1.5 liters/hr) and GENIUS®-CHFD (●; 4.2 liters/hr) on *ex vivo* whole-blood production of inhibitory soluble TNF receptor type I (TNFsRI) in septic patients with acute renal failure. Symbols indicate the medians of *N* = 7 for CVVH and *N* = 5 for CHFD.

polyamid membrane and 36 liters of fluid exchange (ultrafiltration vs. substitution) transiently improves suppressed production of TNF-α by approximately 300% after three hours of treatment with a new filter. At the end of a 24-hour treatment, suppression was as severe as at the start of a new filter for CVVH. The ultrafiltrate did not contain significant amounts of suppressing activity, suggesting that the putative suppressing factor is not removed by filtration. The most plausible explanation for the improved production of ET-induced TNF-α production after three hours of CVVH is adsorption of the suppressing factor to the filter membrane. As adsorption is saturable, removal is limited, and suppression increases again. Removal by saturable adsorption has previously been described for circulating cytokines such as IL-1β and TNF-α [4, 5] and for complement components such as factor D [6].

Improvement of suppressed whole blood production of TNF-α was more effective during high-flux hemodialysis using the GENIUS® system. In this system, dialysate circulates in a closed loop connected to a 75 liter tank in which the pressure is slightly positive. Therefore, the mechanism of solute transport across the high-flux membrane is not only diffusion but also filtration combined with backfiltration. During GENIUS®-CHFD, suppressed TNF-α synthesis is improved within the first three hours. It continues to increase until a plateau is reached at six hours, which lasts until the end of the 18-hour treatment.

In parallel to improved TNF-α production in whole blood, suppressing activity appeared in spent dialysate, indicating that during CHFD with polysulfon F60S, a soluble suppressing factor is removed by diffusion and/or filtration. An additional effect caused by adsorption to the dialyzer membrane is possible. Although TNF-α production in septic patients improves significantly during CHFD, it has to be pointed out that it only reaches 30% of that in normal controls, and thus further improvement is desirable. If adsorption is the major mechanism of removal during CVVH with polyamide, one could not expect that increasing the exchange volume (for example, to 72 liter/day) could remove more suppressing activity. In contrast, increasing the dialysate volume in GENIUS-CHFD to 150 liters by exchanging the 75 liter tank once a day could increase the removal of the suppressing factor significantly because of higher clearance. This assumption needs to be confirmed by additional clinical studies.

The nature of the putative suppressing factor is not yet known. Also, the importance of the observed modulation of the cytokine response with respect to patients outcome remains to be determined. However, *ex vivo* ET-induced whole blood production of proinflammatory cytokines may be useful to monitor the effect of CRRT (particularly high-volume CVVH or CHFD) in the treatment of MODS.

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